

## LETTERS TO THE EDITOR

### *X-Ray Diffraction of the Crystalline Structure of the Avian Egg Shell: Some Critical Remarks*

Dear Sir:

We have been involved in a study of the physical properties of the crystalline substance of avian egg shells for the past two years. In this period both optical and x-ray measurements were carried out. On request of our colleagues in the section of Poultry Husbandry and Animal Physiology we had to investigate, among others, the orientation of the calcite crystals in the shell.

In performing these measurements we proceeded in much the same way as investigators in the field of petrofabrics. Measurements of the orientation of calcite crystals carried out with a petrographic microscope equipped with a universal stage showed that the calcite crystals are more or less randomly arranged. In all the cases observed the c-axes of the crystals are highly oblique to the shell's surface.

About two months ago our attention was drawn by the conclusions of Cain and Heyn (1964). According to these investigators the crystallographic c-axis is only 16 to 28° inclined with respect to the normal of the shell's surface. The conclusion is notably at variance with our preliminary results. Therefore, we decided to settle the dispute by introducing another method. We knew that one of us has some experience with the use of an x-ray texture goniometer.

This goniometer registrates automatically the intensities of x-ray beams reflected by one selected set of lattice planes. In our investigation we choose the important rhombohedral face of calcite 104 ( $10\bar{1}1$ , using rhombohedral indices) and the lattice planes 108 and 110 in order to check our results and to link them up with those of Cain and Heyn, *loc. cit.* During the measurement the specimen is rotated around two axes: one perpendicular to the specimen (360° per 6 minutes) and one parallel with its surface (5° per 6 minutes). Thus the whole diagram of intensities of one set of lattice planes is obtained in about 90 minutes. By the combination of the two rotations, reflections from all these planes of which the poles make angles of more than 15° with the surface of the specimen are recorded.

The next step was simple. We bought a nice large white egg with a rather smooth and regular shell. Part of the shell (20 × 20 mm) was mounted on the goniometer. The selected piece of shell was as flat as possible (strongly curved surfaces are difficult to measure). The results illustrated in three stereograms with the normal to the shell as centre speak for themselves.

The method only studies a thin layer of the outer part of the shell of approximately 0.003 mm. Calcite crystals within this layer are incorporated in the measurement recorded in the stereograms. Research on deeper layers has not been performed as yet. Therefore, the measurements illustrated in the three stereograms are the result of the contribution of at least some 6000 calcite crystals in the outer part of the shell fragment. Readers interested in this method may find useful references in the application data made available by the firms producing these x-ray goniometers.

The results of our preliminary x-ray work corroborate our optical measurements. The pole on 104 is rigorously parallel with the normal of the shell's surface. Conse-

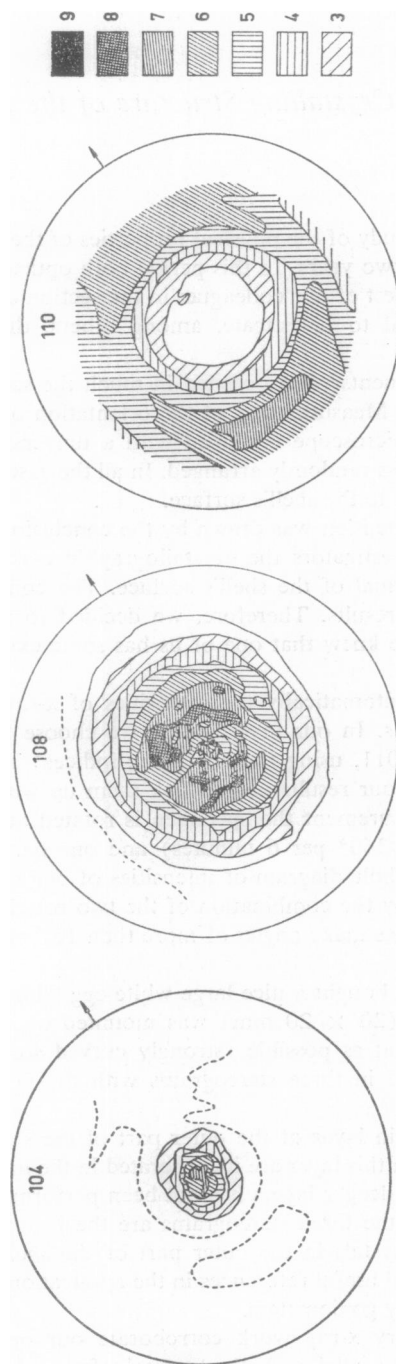


FIGURE 1 Three stereograms illustrating the distribution of poles of calcite planes 104, 108, and 110, as recorded from the outer side of an avian egg shell. The intensity is recorded in arbitrary units as given in the legenda. Siemens x-ray texture goniometer; Cu radiation  $360^\circ/6$  minutes and  $5^\circ/6$  minutes: sample size  $20 \times 20$  mm. The arrow indicates the direction of the longest axis of the egg.

quently the calcite crystals are arranged in such a way that the rhombohedral face is parallel with the egg's surface. The spread is only  $20^\circ$ . The angle between the c-axis of calcite and the pole on 104 is about  $45^\circ$ . Hence the c-axes are arranged in a cone with an apex of  $90^\circ$ . C-axes are oblique to the shell's surface with an average angle of  $45^\circ$ .

In order to check whether the other planes (and therefore also the c-axes) possess a preferred orientation or are randomly oriented (as far as this is permitted by their 104 planes parallel to the surface of the egg), the distribution of the poles of the 110 plane, parallel with the crystallographic c-axis, was determined. From the 110 stereogram it follows that the poles of that plane are oriented nearly at random in a cone with an apex of  $120^\circ$ . There is a slight preferential distribution in the direction of the longest axis of the egg shell. The angle between the poles of 104 and 110 in calcite is about  $60^\circ$  and consequently the obtained 110 diagram is just the one that would be expected. The angle between the poles of 104 and 108 is about  $18^\circ$ . The incomplete girdle on the 108 diagram belongs to a cone with a somewhat larger apex (about  $30^\circ$ ). This inconsistency is probably at least partly caused by the spread of 104 axes discussed above. The weak tendency towards preferential orientation in the direction of the egg's longest axis is seen also in the 108 stereogram.

In conclusion we must state that our results on only a few specimens are at variance with the conclusions drawn by Cain and Heyn as well as with those of Terepka (1963). The rhombohedral face of calcite is of utmost importance for an explanation of the properties of avian egg shell's, whereas the c-axis of calcite is randomly arranged and on the average  $45^\circ$  inclined to the shell's surface.

## REFERENCES

- CAIN, C. J., and HEYN, A. N. J., 1964, X-Ray diffraction studies of the crystalline structure of the avian egg shell, *Biophysic. J.*, **4**, 23.  
TEREPKA, A. R., 1963, Structure and calcification in avian egg shell, *Exp. Cell Research*, **30**, 171.  
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